Altered Responsiveness to Ovarian Steroids in the Mammary Glands of Female BALB/c Mice Neonatally Exposed to DES

Mikiko Asai, Russell C. Hovey, Nira Colyn, Erika Ginsburg, and Barbara K. Vonderhaar.
Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD.

Neonatal exposure of mice to DES, which results in long-term abnormalities in the reproductive tract and mammary glands, serves as a model of human *in utero* exposure to DES because of the similarity in developmental stage of the reproductive tract and mammary glands of the mouse neonate and the human fetus. In this study, we investigated the long-term morphological abnormalities in the mammary glands of female BALB/c mice neonatally treated with DES. Particularly, we focused on the relationship between these abnormalities and alteration in the responsiveness to ovarian steroids. Within 36 hours after birth, female mice were given a single s.c. injection of various doses of DES (0.0125, 0.125, 1.25, 12.5, 25, 50mg/pup) in sesame oil vehicle, or sesame oil (SES) only as control. The mammary glands were examined at 12weeks of age. Two distinct morphologies were observed; dilated ducts or extensive lobulo-alveolar development (LAD) in the absence of dilated ducts. The LAD score of the mammary glands of mice treated with 0.0125mg of DES was significantly higher than SES controls and the score gradually decreased with increasing doses of DES. Inversely, the incidence of dilated ducts was 5% in mice treated with 0.0125mg of DES and increased linearly with increasing doses of DES. Therefore, the two characteristic morphologies observed in the mammary glands are inversely correlated. Examination of the ovaries in these mice showed that the mice demonstrating extensive LAD had corpora lutea while those with dilated ducts had no corpora lutea regardless of the DES dose given. Thus, we hypothesized that anestrus plays an important role in the appearance of dilated ducts and that neonatal exposure to DES hypersensitizes the mammary glands to ovarian steroids. In order to test this hypothesis, animals neonatally treated with DES 12.5mg were subsequently ovariectomized (ovx) at 5 weeks of age and beginning at 6 weeks, the animals were treated with estrogen (E), progesterone (P) or their combination for 2 weeks. DES treated mice given E after ovx had more severe dilated ducts than SES treated mice given E, demonstrating that animals neonatally treated with DES are more sensitive to E than controls. However, administration of P in addition of E after ovx prevented the appearance of dilated ducts, suggesting that anestrus is essential for the appearance of these structures. DES treated mice given only P after ovx had more side branching and a higher LAD score than SES controls treated with P indicating P responsiveness in the absence of E. The expression of estrogen receptor (ER) and progesterone receptor (PR) mRNA in the mammary glands was examined by RT-PCR. The expression of ER or PR was correspondent to the responsiveness to E or P by the mammary glands of the ovx mice.

DES Induces Neoplastic Transformation of Human Breast Epithelial Cells *in vitro*

Yun Fu Hu, Irma H. Russo, Carolyn M. Slater, and Jose Russo. Breast Cancer Research Laboratory, Fox Chase Cancer Center, Philadelphia, PA.

Prolonged exposure to estrogens is associated with an increased risk of developing breast cancer. However, what roles, if any, estrogens play in the etiology of human breast cancer is largely unclear. To determine the carcinogenicity of DES in human breast, MCF-10F cells, which are *bona fide* normal human breast epithelial cells expressing nearly all genetic, cytogenetic, ultrastructural and phenotypic characteristics of normal breast epithelium, were treated with 1.0 mg/ml DES for 24 hrs twice weekly for up to 7 weeks. The cells treated for a week were either passaged for another round of treatments or seeded in agar methocel and cultured for 21 days to determine survival efficiency and colony efficiency indicative of anchorage-independent growth. MCF-10F cells treated with chemical carcinogen benzo[a]pyrene (BP) using the same regimen were used as positive controls, while solvent-treated cells served as negative controls. While the survival efficiency was maximum at 55.5≻≺29.6% after 7 weeks of DES treatments, the colony efficiency of DES-treated cells reached its peak after 2 weeks of treatments (0.484≻≺0.159%) and did not increase any further after longer treatments. In contrast, both survival and colony efficiencies in BP-treated cells were increased with prolonged exposure. Survival efficiency in cells treated with BP for 2, 4 and 7 weeks were 17.4–7.0%, 47.7≻≺21.5%, 407.0≻≺39.0%, respectively, and colony efficiency in cells treated with BP for 2, 4 and 7 weeks were 0.794≻≺0.124%, 1.228≻≺0.281% and 15.525≻≺0.538%, respectively. A total of 112 colonies, chosen at random, were then isolated from cells treated with DES or BP for 1, 2 or 7 weeks and expanded *in vitro*. The percentages of colonies survived in subsequent cultures were 0% (0/8), 0.1% (2/24) and 100% (24/24) for cells treated with DES for 1, 2 and 7 weeks, and 0% (0/8), 0.29% (7/24) and 100% (24/24) for cells treated with BP for 1, 2 and 7 weeks, respectively. These data indicated that the survival and expandability of the isolated colonies were highly correlated with the duration of treatments regardless whether DES or BP was used. Tumorigenicity of 14 expanded colonies, selected at random, was determined by inoculation of $2x10^7$ cells into the mammary fat pad of 62 SCID mice. Whereas the parental MCF-10F cells were not tumorigenic, all the transformed colonies were tumorigenic, forming tumors in 33.3%-66.7% SCID mice within 4 weeks. All the tumors grew very fast for the first ten days reaching up to 5 mm and then regressed. DNA fingerprinting analysis on the tumor cells verified their origin of MCF-10F cells. Microarray cDNA analysis showed that expression of a large number of genes was either increased (e.g. sulfotransferase, thiosulfate sulfotransferase, hydroxy-delta-5-steroid dehydrogenase, steroid receptor coactivator-1, tyrosine phosphatase) or decreased (e.g. SNC tumor suppressor gene, annexin IV, firbrinogen β-chain precursor, leukocyte elastase inhibitor, phosphalipases Cγ2 and Cβ4) in the DES-transformed cells as compared to their parental MCF-10F cells. In conclusion, our results indicated that multiple treatments of MCF-10F normal human breast epithelial cells with DES induce malignant neoplastic transformation and alterations in the expression of many genes. Since MCF-10F cells are estrogen receptor-negative, our data provide the first experimental evidence that DES can initiate human breast cancer, possibly through a mechanism involving direct DNA damage (supported by grant R01 CA67238 from NIH).

Increased Tumor Prevalence in DES-lineage Mice

Retha R. Newbold, Wendy N. Jefferson, Rita B. Hanson, E. Padilla-Banks, Bill C. Bullock, and J.A. McLachlan, Laboratory of Toxicology, Environmental Toxicology Program, NIEHS, Research Triangle Park, NC; Department of Pathology, Wake Forest University School of Medicine, Winston-Salem, NC.

Prenatal exposure to diethylstilbestrol (DES) is associated with reproductive tract abnormalities including subfertility and neoplasia in experimental animals and humans. Our recent studies describe an increased susceptibility for tumors to be transmitted from developmentally DES-exposed female mice to their female descendants (Newbold et al., 1998). To evaluate the possibility that adverse effects may also be transmitted to their male descendants, outbred CD-1 mice were treated with DES during three stages of differentiation: Group I was treated on days 9-16 of gestation (2.5, 5 or 10 μg/kg maternal body wt.) during major organogenesis; Group II was treated once on day 18 of gestation (1000 μg/kg maternal body weight) just before birth; and Group III was treated on days 1-5 of neonatal life (0.002 μg/pup/day). DES-exposed female mice (F1) in each group were raised to maturity and bred to control males to generate DES-lineage (F2) male descendants. Reproductive performance of F2 males when bred to control females was not different from unexposed males. However, in F2 males sacrificed at 17-24 mos. of age, an increased incidence of reproductive tract tumors including rete testis tumors was seen. Because tumors were seen in all three DES developmental treatment groups, all exposure periods were considered susceptible to perturbation by DES. These data suggest that although fertility of the DES F2 male mice was unaltered, increased susceptibility for tumors was transmitted from the DES “grandmothers” to subsequent generations. Alterations in expression or structure of target genes, previously shown to be regulated by estrogens, are under investigation. Using this animal model, we can now systematically analyze and detect changes caused by DES, which will enable us to compare similarities and differences between mice and humans. The ability to detect these genetic/epigenetic changes may represent an important advancement in future cancer therapy and prevention. Furthermore, this mouse model permits us to reach across species and learn more about mechanisms involved in cancer, in particular, the factors underlying the genetic predisposition to cancer.

Use of an ERE-*Lac Z* Transgenic Mouse to Assess Tissue Specificity of Estrogens During Development and in Adulthood

Susan C. Nagel and Donald P. McDonnell, Department of Pharmacology and Cancer Biology, Duke University Medical Center, Durham, NC.

The estrogen receptor (ER) is a ligand inducible transcription factor expressed not only in the reproductive tract but also in the brain, in bone and in the cardiovascular system. Steroidal estrogens, the endogenous ligands for ER, are required for normal development and in multiple systems in adulthood. However, a comprehensive characterization of how, when, and where these ER-mediated actions take place has not been accomplished. Toward this end we have undertaken a transgenic approach that will allow for the detection of individual cells that contain activated ER throughout the body. We designed a reporter transgene, containing three copies of the vitellogenin estrogen response element linked to a minimal thymidine kinase promoter and the reporter gene *lac Z*. This reporter construct was estrogen inducible in tissue culture and showed differential responsiveness to estradiol in three cell lines. Half-maximal estrogenic stimulation of §–galactosidase activity was observed at 5.7 pM ± 1.7, 103.3 pM ± 73.3, and 16.0 pM ± 3.3 in the human derived MCF-7 and HepG-2 cell lines and the mouse derived NIH 3T3 cell line, respectively, using both endogenous and transfected ER. The synthetic estrogens diethylstilbestrol and clomiphene showed half-maximal stimulation of this reporter construct in MCF-7 cells at 20 pM and 30 nM, respectively. Following microinjection of one cell fertilized eggs and implantation of these zygotes into pseudopregnant female mice, three transgenic mice were identified out of 62 pups. After breeding these founders to obtain enough transgenic animals, they will be examined for the global distribution of tissues that exhibit ER agonist activity in response to estrogens. We believe this ER indicator mouse will aid in understanding the tissue specific mechanisms of estrogen action, in the localization of activated ER during development, and the identification of potentially novel sites or mechanisms of action of both steroidal and xenobiotic estrogens, such as diethylstilbestrol.

Low Dose Effects Following Prenatal Exposure to Estrogen

Kristina Thayer[1], Kembra Howdeshell[2], Rachel Ruhlen[4], Fred vom Saal[2], Dennis Lubahn[3], David Buchanan[5], Paul Cooke[5], Wade Welshons[4]. [1]Department of Anatomy, University of California, San Francisco, CA, [2]Division of Biological Sciences [3]Departments of Biochemistry and Child Health and [4]Department of Veterinary Biomedical Sciences, University of Missouri, Columbia, MO, [5]Department of Veterinary Biosciences, University of Illinois, Urbana, IL.

We examined the effects of prenatal exposure to low doses of estrogens on reproductive organs in male and female mice. In the first experiment, our objective was to examine the consequences for offspring resulting from maternal ingestion of clinically relevant doses of a synthetic estrogen found in many oral contraceptives (OCs), 17a-ethinyl estradiol (EE2), since millions of women continue to take OCs while pregnant. In the second experiment, we examined the effects on reproductive organs resulting from maternal ingestion of another synthetic estrogen, diethylstilbestrol (DES), in offspring with 3 genotypes: mice lacking an allele (—) for estrogen receptor alpha (ERa), animals heterozygous for this mutation (+-), and wild-type (++) animals.

In the first experiment, pregnant mice were fed clinically relevant doses of EE2 ranging from 0.002-2 mg/kg body weight on days 0-17 of pregnancy. The male offspring were examined at 2 and 5 months of age with regard to reproductive organ weights and daily sperm production. The results of the experiment with EE2 showed that prenatal exposure resulted in an increase in prostate weight at both 2 and 5 months. In addition, there were significant decreases in daily sperm production in all dose groups when males were 2 months old. However, when examined at 5 months of age, daily sperm production did not differ in control and treated mice.

In the second experiment, pregnant dams heterozygous for a mutation in the ERa gene were fed doses ranging from 0.02-20 mg/kg of DES on days 11-17 of pregnancy. Female offspring were ovarectomized and implanted with a 5 mg estradiol capsule at 6 months of age and the uterus, liver, kidney, and spleen were weighed. In response to the same dose of estradiol in adult life (to eliminate variation due to estrous cycles), there was an inverted U shaped dose-response in wild-type females as a function of prenatal DES dose. Females that were exposed to lower prenatal doses of DES had increased uterine weight, whereas animals exposed to the highest prenatal DES dose had reduced uterine weight. In a subset of tissues, epithelial cell height was measured, but was not influenced by prenatal DES treatment. Interestingly, this pattern of uterine weight response was similar to that of the prostate in CF-1 male mice exposed to a similar range of DES doses. Spleen weights in wild-type females was also affected at low doses, significantly increased in the 0.02-2mg/kg group; spleens in the highest dose was also larger than controls, but this increase was not statistically significant. Wild-type female kidney and liver weight were also increased in some, but not all DES treatment groups. None of these effects were present in either heterozygous or ERa knockout females. In contrast, male offspring did not appear to be as affected as the females. In males, there was no statistical change in reproductive organ weights in relation to prenatal DES treatment in any genotype. This included prostate weight, a measure we have found to be sensitive to DES in CF-1 mice. Kidney, spleen and liver weights were also not affected in male offspring. There was, however, a general decrease in daily sperm production in heterozygous males across DES doses. Overall, these results indicate that low doses of estrogen during prenatal life can have long-term consequences for the offspring, although the detection of these effects is complicated by the presence of strain differences and time-points when responses are measured.

Are DES-exposed Persons Whose Mothers had Multiple Spontaneous Abortions at Increased Risk?

Jan Bernheim[1], Bente Kaiser[1], Guido Goelen[2]. [1]Department of Human Ecology and [2]Oncologisch Centrum, Free University of Brussels Medical School, Brussels, Belgium.

Background

The registered rise and fall between the 1960's and 1990's of the incidence of CCA was in phase with the estimated time course of the exposure of the population to DE until 1971 (Mellnick e.a., N.E.J.M., 1987; 316:514), and constitutes evidence for a casual role of DES. Philip Cole and Nathan Mantel have in 1985 suggested an additional hypothesis: that DES would have rescued just those otherwise doomed fetuses who, (in contemporary terms, because of e.g. defects in onco-developmental genes), carried a high risk of CCA.

Hypothesis

Several recent lines of evidence suggest that this hypothesis deserves renewed attention.

1. In the 3 controlled trials of antenatal DES treatment with published M/F sex ratio outcomes, they ranged from 1.16 to 1.38 in the DES arm. This suggests that DES (contrary to other xenoestrogens!) may rescue male and/or imperil female fetuses.
2. Some mechanisms by which high estrogens might in some cases salvage endangered fetuses have been identified.
3. Though on large samples of unselected pregnancies DES was not proven effective (and maybe somewhat detrimental), there are a few anecdotal cases of (in 1 case consistent) apparent effectiveness in habitual abortion on record. However, most are probably unpublished. These anecdotal cases contributed to the popularity of DES prophylaxis in the 50's and 60's.
4. One such case we are studying currently suggests an onco-developmental problem: the daughter of a G11Ab10P1 mother (9 first and 1 second trimester miscarriages), born after DES, had ovarian carcinoma. So did her later born non DES-exposed sister. The mother had thyroid and breast cancer, and there were multiple cancer cases, including one of male breast cancer in the family.
5. The expression of the Wnt7a gene involved in female genital tract development is experimentally disrupted by DES in rodents. It may be an onco-developmental gene, and, just as RU-486 (Mifepristone) can be an agonist on a point-mutated progesterone receptor, DES could, depending on the context, favor fetal salvage or be abortive.

According to the Cole-Mantel hypothesis, DES-exposed women born of mothers with multiple abortions would be at increased risk of CCA and possibly other cancers. Indeed, a maternal history of D1 prior spontaneous abortion (RR2.4) is (after first trimester exposure (RR=4) the strongest risk factor for CCA among DES-exposed young women (Herbst e.a., Am. J. Obstet. Gynecol., 1986; 154:814).

Questions

l. Should special efforts be made to identify those persons who were born after a possible rescue by DES treatment in order to:
   A. Ensure that they are offered adequate surveillance?
   B. If consenting, study them and their families for onco-developmental abnormalities?
2. Do the potential benefits for themselves and for our understanding of carcinogenesis outweigh the quite possibly unjustified alarm?
3. If not, should (elderly) gynecologist who prescribed DES be contacted so as to allow them to "paternalistically" evaluate the ratio of risks and benefits for their (former) patients? If so, what would be the most appropriate way to contact them?

Is There Evidence for Estrogen Imprinting in Humans? Midlife Status of Health of Antenatally DES Exposed Persons (MISHADE)

Jan Bernheim[1], Diane Anderson[5], Jean-Pierre Daures[3], Laurent Giraudon[3], Elizabeth Hatch[4], Arthur Herbst[5], Bente Kaiser[1], Leon Kaufman[2], Raymond Kaufman[6], Levi Wijns[1]. [1]Department of Human Ecology, Free University of Brussels Medical School, Brussels, Belgium, [2]Department of Statistics, Free University of Brussels Medical School, Brussels, Belgium, [3]Institut de Recherches Cliniques, University de Montpellier, France, [4]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [5]Department of Obstetrics and Gynecology, University of Chicago, [6]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX.

Rationale

In rodents, experimental high estrogen exposure during fetal development can result in lifetime epigenetically altered expression of several estrogen-mediated functions, and alters the skeleton. This phenomenon has been called “estrogen imprinting”. On the other hand, hormonal replacement therapy with estrogens (HRT) has been shown to reduce cardiovascular and osteoporosis risks. Estrogens also play a role in CNS development and the prevention of Alzheimer’s disease. If estrogen imprinting also occurs in humans, this is likely to be demonstrable in antenatally diethylstilbestrol (DES)-exposed subjects, who may have a different height and in older age may have altered risks for the above estrogen-influenced conditions.

Hypotheses

1. There is evidence of estrogen imprinting by antenatal exposure to DES in 50 year olds, manifesting as altered values of physiological and health-related variables which are influenced by estrogens.
2. These effects are limited to subjects whose exposure to DES started before some pivotal fetal maturity age.

Proposed MISHADE Study

Comparison of DES-exposed and control cohorts “Dieckmann”-Chicago, “Vessey”-U. C. London, DESAD) for parameters potentially influenced by estrogen imprinting, including reproductive history, height, bone density, cardiovascular and Alzheimer risks. We hope to present preliminary power and feasibility calculations on the basis of the already Chicago-cohort data, and invite those able to contribute to a MISHADE study join us in an ad hoc post-workshop investigators meeting after 12:30 on Tuesday 20th.

Report of the Netherlands Registry of Clear Cell Adenocarcinoma (CCA) Preliminary Data of the 1999 Update

Antonius Hanselaar[1], Erik Boss[2], Hebste Shirango[1], Peter de Wilde[1], Jan Bernheim[3]. [1]Department of Pathology, [2]Department of Gynecology and Obstetrics, University Hospital Nijmegen, The Netherlands, [3]Department of Human Ecology, Medical School, Vrije Universiteit Brussels, Belgium.

DES was prescribed between 1947 and 1975 mainly in the USA, France and the Netherlands. In 1971, a first case control study by Herbst et al indicated a relation between CCA and exposure to exogenous oestrogens *in utero*. Other risk factors for CCA were threatened abortion or a history of spontaneous abortion (for which DES was prescribed). In the USA 600 cases were registered on a voluntary basis, mainly in the seventies. DES exposition in this group was about 60%. It seemed that the estimated increase and decrease of previous DES exposure of the population was in phase with CCA incidence in young women in the USA. Melnick et al estimated the absolute risk for DES daughters to develop CCA at about 1:1000.

The causal relationship between antenatal DES exposure and CCA has been questioned because other epidemiological criteria of causality are not fulfilled. For instance, the incidence of CCA in young women in the USA appears not higher than in Norway, where DES was not prescribed. The also bell-shaped secular evolution of the incidence of non-DES related CCA in the USA is similar to that of DES related cases, suggesting some other risk factor that was contemporary with DES.

The Netherlands offers exceptional opportunities for the study of CCA as a special case or model of hormonal carcinogenesis. It has a longstanding population-based pathology registry (PALGA), from which the Central Netherlands Registry (CNR) for CCA draws its cases, and a widespread usage of DES. Previous registry updates have shown the age distribution of patients with CCA in the Netherlands to be bimodal, with as many post-menopausal (non-DES exposed) patients as premenopausal. Also, non-DES exposed cases show a prominent incidence peak around age 25. This suggests genetic susceptibility, other exogenous risk factor(s), and/or a promoting role of both menarche and menopause. Factors like onco-developmental genes and other xeno-oestrogens may be important for the understanding of hormonal carcinogenesis.

Preliminary results of the presently ongoing registry update of women with CCA born after 1947 show, that CCA in DES exposed women is significantly ($p<0.001$) more often vaginal, compared to non-DES exposed women. Over the years, the average age at diagnosis of CCA cases reported to the CNR has been increasing. This was significant ($p<0.001$) more often vaginal, compared to non-DES exposed women. Over the years, the average age at diagnosis of CCA cases reported to the CNR has been increasing. This was significant ($p<0.001$) for the DES exposed women with vaginal CCA. A further matter of major interest is whether the bell shaped secular incidence rate that was described in the USA will also be observed in the Netherlands.

Cancer Risk in Women Exposed to Diethylstilbestrol *in utero*

E.E. Hatch[1], J.R. Palmer[2], L. Titus-Ernstoff[3], K.L. Noller[4], R.H. Kaufman[5], R. Mittendorf[6], S.J. Robboy[7], M. Hyer[8], C.M. Cowan[6], E. Adam[5], T. Colton[2], P. Hartge[1], R.N. Hoover[1]. [1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [2]Slone Epidemiology Unit, Boston University School of Public Health, [3]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [4]Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, [5]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [6]Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, [7]Departments of Pathology and Obstetrics and Gynecology, Duke University Medical Center, Durham, NC, [8]Information Management Services, Rockville, MD.

To estimate directly the risk of adult malignancies related to *in utero* exposure to diethylstilbestrol (DES), we attempted to reassemble, combine, and update all of the U.S.-based cohorts established in the 1970's to study this exposure. We were able to identify 4536 DES-exposed daughters and a comparison group of 1544 unexposed. Over half of the subjects were originally identified by medical record review, and the remainder had documented exposure (or non-exposure) to DES. We traced these individuals, sent them a questionnaire, and obtained pathology reports and death certificates for those with an indication of a malignancy. We compared the frequency of cancer in each group to that "expected" based on age and time-specific rates from population-based tumor registries via standardized incidence ratios (SIR). We also compared directly the rates in the exposed to those in the unexposed via Rate Ratios (RR). We calculated 95% Confidence Intervals (CI) for each of these measures. So far, DES-exposed daughters have not experienced an increased risk for all sites (RR, 0.96; CI, 0.58-1.56) or for individual cancer sites, except for clear cell adenocarcinoma of the vagina. Three such cases occurred among the exposed (SIR, 40.7; CI, 13.1-126.2), leading to an overall estimate of the attack rate of 1.5 cases per 1000 exposed. This estimate is remarkably close to the rate of 1 per 1000 estimated from population data in 1987. The RR for breast cancer was 1.18 (CI, 0.56-2.49); adjustment for known risk factors did not alter this result. The results should be reassuring to the DES-exposed population, since they confirm previous estimates that CCA is an uncommon occurrence and they also reveal no major excesses in risk of other types of cancer. Since the majority of women included in our study are currently younger than 50 years, it will be important to continue follow-up of the cohort to monitor cancer risk as the cohort ages.

Incidence of Cervical Dysplasia in DES-exposed Daughters: Update and Long-term Follow-up of the DESAD Cohort

E.E. Hatch[1], R.N. Hoover[1], A.L. Herbst[2], K. Noller[3], R.H. Kaufman[4], L. Titus-Ernstoff[5], J.R. Palmer[6], M. Hyer[7], S.J. Robboy[8]. [1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [2]Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, [3]Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, [4]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [5]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [6]Slone Epidemiology Unit, Boston University School of Public Health, [7]Information Management Services, Rockville, MD, [8]Departments of Pathology and Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.

The prevalence of squamous cell cervical and vaginal dysplasia was similar between DES exposed and unexposed daughters during the initial phase of the DESAD study, yet a later study of incidence reported a nearly two-fold increased risk of dysplasia in the exposed. The purpose of the current study was to determine whether the incidence of cervical dysplasia in DES exposed daughters has remained elevated since 1982 when the last follow-up of the DESAD cohort ended.

We analyzed data on 3299 exposed and 869 unexposed daughters, after excluding cases of dysplasia diagnosed prior to 1982, subjects who had a treatment of the cervix before 1982 that could have altered the subsequent risk of dysplasia, and subjects who had had a hysterectomy. The analysis focused only on cases of moderate or severe dysplasia (CIN2 +) diagnosed by biopsy. All cases included were confirmed by pathology report, and 71% were also reviewed centrally by one pathologist. Poisson regression analysis was used to compute relative risks (RR) and 95% confidence intervals (95% CI) adjusting for age, calendar year, and other covariates. Follow-up began in 1982 and continued through 1995.

The RR (95% CI) among the DES exposed compared to unexposed, based on 78 cases of CIN2 + confirmed by pathology report, was 1.52 (0.80,2.89), adjusting for age and calendar year of diagnosis. Similar results were found among subjects originally identified by record review versus those who were self- or physician-referred. When the analysis was restricted to the 2448 exposed and 651 unexposed daughters who participated in regular screening during the clinical examination phase of the DESAD project (1975-1981), the RR among the exposed was reduced (RR=1.19; 95% CI=0.60-2.36). RRs were slightly higher when cases without centralized slide review were excluded. Preliminary findings controlling for potential confounding variables and frequency of cervical cancer screening will also be presented.

Methods and Preliminary Findings from the NCI DES Follow-up Study, Based on Responses to Mailed Questionnaires

E.E. Hatch[1], L. Titus-Ernstoff[2], J.R. Palmer[3], K.L. Noller[4], R.H. Kaufman[5], A.L. Herbst[6], M. Hyer[7], P. Hartge[1], R.N. Hoover[1]. [1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [2]Slone Epidemiology Unit, Boston University School of Public Health, [3]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [4]Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, [5]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [6]Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, [7]Information Management Services, Rockville, MD.

Beginning in 1993, NCI in collaboration with DES researchers at five field centers, identified, traced, and sent questionnaires to over 6000 daughters and 3000 sons from several different cohorts studied in the past. We also attempted to identify and include subjects who had not previously been followed, by contacting offspring of mothers who had participated in a large study of the possible relationship between breast cancer and DES exposure during pregnancy. We obtained 5723 questionnaires from daughters and 2746 questionnaires from the sons, for a response rate of 88% among females and 75% among males. Pathology reports were sought to confirm reports of cancer, benign breast disease, and cervical and vaginal dysplasia. Selected characteristics of respondents will be summarized, including sociodemographic characteristics, health screening behaviors, and prevalence of selected diseases and conditions reported on the questionnaires.

Exposed and unexposed subjects were reasonably comparable in terms of sociodemographic characteristics. Both DES-exposed daughters and sons tended to have higher levels of education than the unexposed subjects (approximately 62% of both exposed daughters and sons had at least a four year college degree in contrast to 53% of unexposed offspring). Over 95% of the cohort was white; and current marital status was similar in the two groups.

DES exposed daughters tended to have more health screening examinations of most types than unexposed subjects. For example, 21% of exposed daughters versus 10% of unexposed daughters reported having Pap smears more often than once per year, and 50% of exposed vs. 34% of unexposed reported having at least one colposcopy during the last five years. The frequencies of mammograms and breast examinations were similar among the two groups. DES exposed and unexposed sons reported similar frequencies of general physical exams, urologic and rectal exams, and colonoscopies.

There was little difference in the reported prevalence of mental illnesses, sexual orientation, or handedness among DES exposed daughters. However, slightly more DES-exposed sons reported being left-handed (14%) than unexposed sons (11%).

The prevalence of selected autoimmune diseases, genital infections, and other conditions reported on the questionnaires, as well as plans for more substantive analyses of certain outcomes, will also be presented.

Genetic Profile of Breast Cancers in Women Exposed *in utero* to Diethylstilbestrol (DES)

Pamela Larson[1], Kathleen Rowlings[2], Julie Palmer[2], Carol L. Rosenberg[1,3]. [1]Cancer Research Center, [2]Slone Epidemiology Unit, [3]Department of Medicine, Boston University.

Breast cancer is caused by an interaction between genetic, environmental and hormonal factors. Because breast tissue is steroid-hormone responsive and factors related to estrogen exposure are associated with increased breast cancer risk, estrogens have long been suspected to play a prominent role in breast carcinogenesis. The nature and timing of the potentially damaging estrogen exposure is uncertain, however. One speculation is that prenatal exposures may contribute, the paradigm for this being the experience of the several million individuals exposed *in utero*, from the 1940s to the 1960s, to the potent synthetic estrogen diethylstilbestrol (DES). The consequences of exposure to this carcinogen on the estrogen-sensitive female reproductive tract can be severe, including malignancies, phenotypic abnormalities and infertility. The mechanism by which DES causes these abnormalities remains unknown, although *in vivo* and *in vitro* studies and animal models demonstrate that DES exposure leads to multiple cellular toxicities, including genotoxicities, notably abnormalities of chromosome number (aneuploidy) and alterations of microsatellite DNA.

To better understand the effects of prenatal DES exposure, the NCI established the Continuation of Follow-up of DES Exposed Cohort Study, which follows 4000 exposed women and 2000 matched controls. Little is known yet about the effects of DES exposure on other estrogen sensitive tissues, including the breast. Yet the average age of exposed women is 43 years, i.e., approaching the time when the incidence of breast carcinoma begins to rise rapidly. Based on existing data, we hypothesize that breast tumors in women exposed prenatally to DES, in contrast to breast tumors in *un*exposed women, will be characterized by increases in the particular genotoxicities DES causes in *in vitro* or *in vivo* systems: i.e. microsatellite instability (MI) and loss of heterozygosity (LOH), a molecular counterpart of aneuploidy. To test our hypothesis, we will use a technique we developed for examining small quantities of microdissected archival breast tissues to investigate the genetic profile of ~ 40 breast carcinomas occuring in both exposed and unexposed women in the cohort study. This technique utilizes 20 microsatellite markers selected for location at sites relevant to breast tumorigenesis and then multiplexed into only 5 PCRs. We can obtain reproducible results from < 500 cells, making it feasible for investigation of small lesions and control breast tissue from archived blocks. Each tumor's genetic profile, or pattern of abnormalities as revealed by these PCRs, will be determined, and the pattern for exposed vs. unexposed compared. This should indicate particular loci, and perhaps general pathways, by which early aberrant estrogen exposure promotes carcinogenesis in the DES-exposed cohort and, more broadly, in other women as well.

Further Follow-up of the Risk of Breast and Other Cancers in Women Exposed to DES in Pregnancy (DES Mothers) Compared to Unexposed Women

L. Titus-Ernstoff[1], E.E. Hatch[2], R.N. Hoover[2], J.R. Palmer[3], A.L. Herbst[4], K. Noller[5], R.H. Kaufman[6], W. Ricker[7], T. Colton[3], E.R. Greenberg[1], P. Hartge[2]. [1]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [3]Slone Epidemiology Unit, Boston University School of Public Health, [4]Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, [5]Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, [6]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [7]Information Management Services, Rockville, MD.

During the period 1940–1960, diethylstilbestrol (DES), a synthetic estrogen, was given to millions of pregnant women to prevent pregnancy complications and losses. In the 1950's, DES was shown to be ineffective for that purpose; subsequent studies showed increased risks of reproductive abnormalities in exposed offspring and breast cancer in exposed mothers. In this report, we present further follow-up and a combined analysis of cancer risk in two cohorts of women who were exposed to DES during pregnancy. DES exposure status was determined by review of medical records (the Mothers Study cohort), or clinical trial records (the Dieckmann Study cohort). We used Poisson regression analyses to generate relative risks (RR) and 95% confidence intervals (CI) for the relationship between DES and cancer occurrence. DES exposure was assessed in relation to risk of all cancers combined and site-specific cancers. We observed comparably elevated breast cancer risk in each cohort; for both cohorts combined, the RR was 1.27 (95% CI=1.06-1.57), for the Mothers Study cohort, the RR was 1.29 (95% CI=1.06-1.57), and for the Dieckmann cohort, the RR was 1.23 (0.85-1.78). Adjustment for known breast cancer risk factors did not alter these results. Although we noted a slight increased risk of all cancers combined (RR=1.10; 95% CI=0.99-1.23), this was primarily due to the elevated breast cancer risk. We found no evidence that DES exposure during pregnancy increased long term risks of other cancers, including those that may be hormonally mediated. For ovarian cancer the RR was 0.72 (95% CI=0.44-1.17) and for endometrial cancer, the RR was 0.92 (95% CI=0.60-1.39). Our results, in which clinical trial participants have elevated breast cancer risk comparable to that of women whose exposure was ascertained through medical records, suggests that the modest influence of DES on breast cancer risk is real, and not an artifact of possible differences in underlying risk.

Long Term Risk of Cancer in Women Exposed to Diethylstilbestrol *in utero*

Janneke Verloop[1], Marjolein Y.V. Homs[1], Matti A. Rookus[1], Marlies E.T.A. Koster[2], Flora E. van Leeuwen[1].
[1]Department of Epidemiology, The Netherlands Cancer Institute, The Netherlands, [2]Netherlands DES Information Center, Utrecht, The Netherlands.

Exposure to Diethylstilbestrol (DES) *in utero* is known to be related to a strongly increased risk of developing clear-cell adenocarcinoma of the vagina and cervix (CCAC) at a young age. It is not known, however, whether DES-exposure *in utero* is associated with an increased risk of other types of cancer later in life.

We studied the association between DES-exposure *in utero* and cancer risk in a cohort of women registered at the Netherlands DES-Registry, which is based on self-referrals. In 1994, 13.350 DES-daughters received a mailed questionnaire, in which information was obtained on medical history. The questionnaire was returned by 5421 women (response 41%), of which 116 women reported a history of cancer of the vulva, cervix, endometrium, ovary, breast, colon or melanoma. Cancer of the colon and melanoma, for which no clear association with DES was expected, were included as 'marker' tumors to quantify selection bias. CCAC was excluded from analysis. The self-reported information was compared with information from medical files for 96 women (83%). The expected lifetime prevalence was calculated for each type of cancer, based on the prevalence numbers of the regional cancer registry South (IKZ/SOOZ).

The median age of the DES-daughters in this cohort was 30.0 years. A total of 129 cancers were reported by 116 women. The agreement between self-report and information from medical files was poor for cervical cancer (20%) and endometrium cancer (0%). The prevalence ratio (PR) of cervical cancer was significantly increased (PR=6.3; 95% confidence interval (95% CI) 3.4-10.6). No increased PR was found for the other tumors.

In our study we found a slightly increased risk for cervical cancer among DES-daughters. Part of this increase may be due to selection and screening bias. Due to the relatively young age of the DES-daughters, the power of this study was still low, which stresses the need to continue the follow-up of DES-daughters in the future.

Identification of a New Cohort of DES-daughters in the Netherlands

Janneke Verloop[1], Matti A. Rookus[1], Marlies E.T.A. Koster[2], Flora E. van Leeuwen[1]. [1]Department of Epidemiology, The Netherlands Cancer Institute, The Netherlands, [2]Netherlands DES Information Center, Utrecht, The Netherlands.

The effects of *in utero* exposure to Diethylstilbestrol (DES) on cancer risk in adult life are still unknown. The Netherlands is particularly well suited to study cancer risk following DES-exposure *in utero*, because 1) DES has been widely prescribed (an expected number of 110.000 DES-daughters), 2) a registry of DES-daughters, based on self-referrals, was initiated in 1992, and 3) the country has a nationwide cancer registry since 1989.

To study the long-term health effects of DES-exposure *in utero*, we will conduct a retrospective cohort study with a prospective follow-up. DES-daughters will be identified through four sources. The first subcohort (N=10,900) will be identified from a registry of DES-daughters that has been set up in the early nineties by the Netherlands DES Information center. The second subcohort (N=1,500) will consist of DES-daughters screened for clear-cell adenocarcinoma, traced through the Dutch Network and National Database for Pathology. The third subcohort (N=750) will be identified from gynecological records of DES-mothers. The fourth subcohort (N=250) will consist of newly identified sisters of DES-daughters. The control group (N=9,700) will be composed of unexposed sisters of DES-daughters.

Information on DES-exposure, risk factors for hormone-related cancers and on specific gynecological problems will be collected by a short mailed questionnaire. Informed consent will be obtained for both DES-daughters and their mothers.

Eligible cohort members are free of cancer at the start of the follow-up period. The study outcome will be cancer incidence from 1989 onwards up to 2002. The estimated median follow-up will be 10 years, and median age will be 41 years. Cancer incidence in the exposed cohorts will be compared with age-calendar specific incidence rates of the general population (external control group) and with incidence rates in the unexposed cohort (internal control group). The power will be sufficiently high (>85%) to detect a relative risk (RR) of 1.5 for breast cancer, a RR of 2.0 for ovarian cancer and a RR of 3.0 for cervical cancer.

Secondary Prevention of Clear Cell Adenocarcinoma of the Vagina or Cervix in DES Exposed Women

Antonius Hanselaar[1], Erik Boss[2], Leon Massuger[2], Jan Bernheim[3]. [1]Department of Pathology, [2]Department of Gynecology and Obstetrics, University Hospital Nijmegen, The Netherlands, [3]Department of Human Ecology, Vrije Universiteit Brussel, Belgium.

Introduction: The absolute risk for DES-daughters to develop Clear Cell Adenocarcinoma (CCA) has been estimated by Melnick et al at 1:1000. For the purpose of secondary prevention, guidelines have been drawn up that describe different tools in the follow-up for women exposed to DES. Cytologic examination plays an important role in these guidelines, and has been advocated as well as doubted in the literature.

Purpose: To determine the role of cytopathologic examination prior to the detection of vaginal or cervical CCA at the registered patient population in the Netherlands.

Methods: Retrospectively, a systematic collection in the Dutch automated nationwide pathology archive and patients status was performed. All cytologic examinations between 1969 and 1997 within 2 years prior to histologic diagnosis of CCA were studied. Included were women born in the Netherlands after 1947 that are on record in the Dutch population based central registry. Positive cytology was defined as PAP Class III, IV, or V.

Results: Included where 90 patients. CCA location was vaginal in 78%, and cervical in 22% in the DES exposed group (N=50). In the non-DES exposed women (N=38), location was 64% cervical and 36% vaginal. A total of 83 cytologic reports from 49 patients were included. Out of 27 CCA's with (endo) cervical location 85% was preceded by a positive cervical smear. Out of 9 vaginal CCA's 100% was preceded by a positive vaginal smear. FIGO tumor stage I was preceded significantly more frequent by cytologic smears ($p<0.0006$) than the higher tumor stages.

Conclusions: Cytologic examination plays an important role in the early detection of CCA if both cervical and vaginal smear sampling is performed. In a previous study we showed a bimodal age distribution in CCA incidence. One premenopausal (DES related), and one postmenopausal (non-DES related). We assumed a cumulative risk for developing CCA with older age with an increasing risk after DES exposition *in utero*. Cervical and vaginal cytologic follow-up of DES exposed women plays an additive role in early CCA detection. It should not be restricted to young age, but must also be continued now DES-daughters reach menopausal age.

References: Hanselaar A, Loosbroek van M, Schuurbiers O, Helmerhorst TH, Bulten J, Bernheim J. Clear cell adenocarcinoma of the vagina or cervix; an update of the Central Netherlands Registry showing twin age incidence peaks. *Cancer* 1997; 79:11:2229-36.

Pregnancy Outcome in Women Exposed to Diethylstilbestrol *in utero*

R.H. Kaufman[1], E. Adam[1], E.E. Hatch[2], K. Noller[3], A.L. Herbst[4], J.R. Palmer[5], L. Titus-Ernstoff[6], S. Robboy[7], R.N. Hoover[2]. [1]Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX, [2]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, [3]Department of Obstetrics and Gynecology, University of Massachusetts Medical Center, Worcester, MA, [4]Department of Obstetrics and Gynecology, University of Chicago, Chicago, IL, [5]Slone Epidemiology Unit, Boston University School of Public Health, [6]Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center, Lebanon, NH, [7]Departments of Pathology and Obstetrics and Gynecology, Duke University Medical Center, Durham, NC.

Numerous studies have found that DES-exposed daughters tend to be at increased risk of adverse pregnancy outcomes, such as premature delivery, ectopic pregnancy, and second trimester miscarriage. In the current study, we combined data from two previously studied cohorts with documented exposure status, the DESAD and Dieckmann cohorts, and compared the reproductive histories of DES-exposed and unexposed daughters through 1994. All subjects who could be traced were sent a detailed questionnaire in 1994 containing questions related to health history, including information on pregnancies and pregnancy outcome. After two mailings and attempts to administer the questionnaire by telephone, the response rate was 88% for both exposed and unexposed. A total of 3373 exposed and 1036 unexposed were included in the analysis.

Overall, DES exposed daughters were more likely to report an adverse outcome of their first pregnancy. We excluded women who did not become pregnant and those who reported an induced abortion during the first pregnancy. A total of 662 (64%) of the 1032 exposed daughters originally identified by prenatal record review had a full term live birth during the first pregnancy compared to 551 (85%) of the 652 unexposed, after adjusting for maternal age at pregnancy and original center. Similar results were seen in exposed daughters who were originally self-(n=364) or physician-referred (n=648) to the DESAD project. Compared to unexposed daughters, exposed daughters were more likely to have a premature delivery (Relative Risk (RR)=3.06, 95% confidence interval (95%CI)=2.03-4.60). They were also more likely to report stillbirths (RR=3.32, 95% CI=0.73-15.1), spontaneous abortions (RR=2.00, 95% CI=1.54-2.60), and ectopic pregnancies (RR=5.14, 95% CI=2.03-13.0). Similar findings were seen when we analyzed data from any pregnancy.

This study, compared to prior reports, is based on the largest number of women with documented *in utero* exposure to DES, who have been systematically followed throughout much of their reproductive lifetime. Women in the study cohort are now on average 45 years old, therefore, the present analysis may be the last comprehensive study of the reproductive experience of DES exposed women. However, there are still many DES-exposed women within the reproductive age range. Therefore, it is important for the obstetrician-gynecologist to be aware of the possible consequences of *in utero*

Myopia in DES-exposed Amblyopic Subjects

Philip Lempert, Department of Physiology, College of Veterinary Medicine, Cornell University, Ithaca, NY.

Purpose: To evaluate the effect of prenatal exposure to DES on refractive error in an amblyopic population background - Estrogen is closely implicated in neural development and developing neural tissue has a high level of estrogen receptors. Activation of the estrogen receptors inhibits the reproduction of the neural cells. Children with fetal alcohol syndrome have a high incidence of microphthalmos. This has been attributed, in part, to an increase in the estrogen receptors secondary to alcohol exposure. It was therefore assumed that blocking estrogen receptors during pregnancy would result in eyes that are larger than normal. Eyes with increased axial length are typically myopic.

Method: In an effort to test this hypothesis, and to limit the subjects to a clearly defined group, people who had prenatal exposure to DES, an estrogen blocker and had amblyopia were located by way of a notice in DES Action News. They were asked to submit records of eye examinations containing, as a minimum, their refractive errors and visual acuity.

Results: Thirteen DES-exposed amblyopic subjects responded. Their refractive errors are shown in Table 1. The refractive status of the DES exposed group of amblyopes was compared with 255 amblyopic patients with no history of DES exposure. 42 amblyopic subjects with a myopic spherical equivalent in both eyes were identified in the group of 279 amblyopic patients (15 %) It was assumed that this represents the true frequency of myopia among the non DES exposed amblyopic population. The probability that 10 out of 13 amblyopic subjects will be myopic in a sample of amblyopes was found to be $p < 1.0 \times 10\text{-}6$ using the exact binomial test.

These findings suggest that prenatal exposure to DES, which blocks estrogen receptors in developing neural tissue, may be a factor in the appearance of myopia among some amblyopic patients who were born in the two decades beginning in 1950.

Similarly, prenatal exposure to other pseudohormones which are present in some plants and pesticides, may also affect ocular development. This may be related, in part, to the increased worldwide incidence of myopia and the increasing consumption of phytoestrogens such as soy beans.

Lustig RH *In vitro* models for the effects of sex hormones on neurons. *Ann N Y Acad Sci* 1996 Apr 30;784:370–380. Ma ZQ, Spreafico E, Pollio G, Santagati S, Conti E, Cattaneo E, Maggi A. Activated estrogen receptor mediates growth arrest and differentiation of a neuroblastoma cell line. *Proc Natl Acad Sci USA* 1993 Apr 15;90(8):3740–3744. Weiss AH, Kousseff BG, Ross EA. Longbottom Simple microphthalmos. *Arch Ophthalmol* 1989 Nov;107(11):1625–1630. Stromland K (1985): Ocular abnormalities in the Fetal Alcohol Syndrome. *Acta Ophthalmol* Suppl. 171; 63: 1–50. Nasca PC, Liu S, Baptiste MS, Kwon CS, Jacobson H, Metzger BB. Alcohol consumption and breast cancer: estrogen receptor status and histology. *Am J Epidemiol* 1994 Dec 1;140(11):980–8. McEwen BS. Steroid hormones and brain development: some guidelines for understanding actions of pseudohormones and other toxic agents. *Environ Health Perspect* 1987 Oct;74:177–184. Siegel S. Nonparametric Statistcs for the Behavioral Siences: New York : McGraw Hill, 1956:36–42. Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* 1996;87(5 Pt 2):897–904.

Sexual Functioning and Mood among Long-term Survivors of Clear Cell Adenocarcinoma

A.K. Matthews[1], J.E. Aikens[1], S.P. Helmrich, D.D. Anderson[2], S.E. Waggoner[2], and A.L. Herbst[2].
[1]University of Chicago, Department of Psychiatry, [2]University of Chicago, Department of Obstetrics and Gynecology.

Although establishing sexual relationships is viewed as an important developmental milestone for adults, sexual functioning has been a neglected area of investigation for gynecologic cancer survivors. The epidemiology and natural history of diethylstilbestrol (DES) exposed and unexposed clear cell adenocarcinoma (CCA) of the vagina or cervix provides an opportunity for analysis of the important issues faced by gynecologic cancer survivors. Study objectives were to provide a description of the long-term effects of CCA, with specific focus on sexual functioning as a quality of life concern. Further aims were to describe the psychological last effects such as depressive symptoms, and to explore the relationship between health status, coping behavior, social support variables, and depressive symptoms. Participants were 220 long-term survivors (< 5 years) of CCA recruited from the Registry for Research on Hormonal Transplacental Carcinogenesis. Data were collected as part of a larger epidemiological investigation of CCA risk factors and complications. The survey instrument consisted of 105 items regarding gynecologic and sexual complications, psychosocial issues, and other general concerns related to their illness. Participants averaged 41 years of age, and tended to be married Caucasian women with a two-year college degree. Eighty-three percent (n=217) had a report of DES exposure *in utero.* In general, the CCA survivors described their current health in favorable terms. However, 22% reported that CCA interfered with daily activities, social functioning, or personal goals. Sexual dysfunction was common, with 85% of participants reporting at least one category of dysfunction. The most common problems included painful intercourse (38%), lubrication difficulties (47%), inorgasmia (21%), intercourse fears (28%), and reduced sexual drive (33%). Medical/anatomical problems (e.g., vaginal reconstruction) were mentioned by 58% of participants and there was a strong tendency for sexual effects to co-occur with medical effects ($X^2 = 76.2$, $p < .001$). Compared to women with no current sexual problems, women with current problems were more likely to rate their health less positively (Mann-Whitney $U = 3810$, $p < .001$), report moderate to severe levels of depressive symptoms (11% vs. 4%, $X^2 = 4.57$, $p < .05$), and to utilize nonprescription or recreational drugs as coping strategies ($U = 4819$, $p < .05$). Group differences in perceived social support were not observed. Although chronic sexual dysfunction is a legitimate concern by itself, the findings suggest that sexual difficulties are associated with diverse problems in the areas of mood, quality of life, and adjustment. Further work is needed to define the complex associations influencing sexual and emotional adjustment in this population.

Stromal Mucin as a Feature of Cervical and Vaginal Non-clear Cell Adenocarcinomas in DES Exposed Women

Debbie Wu, Gregory Spiegel. Department of Pathology, Roswell Park Cancer Institute.

Background: While cervical and vaginal clear cell carcinoma has been associated with *in utero* exposure to diethylstilbestrol (DES), no studies have been undertaken of the clinicopathological features of cervical and vaginal non–clear cell adenocarcinomas (non-CCCa) in DES exposed women. A review of 7 cases of non–CCCa in DES exposed women raises the possibility that these carcinomas may be associated with a distinct histological feature, *viz.*, a mucinous element with ruptured neoplastic glands with subsequent release of mucin into the cervical myometrium (stromal mucin).

Design: The histological features of 6 cases of cervical and 1 vaginal non-CCCa in DES exposed women were compared with those of 98 cases of cervical non–CCCa that had been entered into Gynecologic Oncology Group (GOG) protocols 128 (31), 085 (11), 120 (15), 123 (22), 141 (12) and 165 (7). The DES history was not stated in 78 cases and reported as negative in the remaining cases. The presence or absence of stromal mucin in a non-irradiated tissue specimen was recorded for each case.

Results: The ages of the 7 DES exposed women were 28, 29, 40, 42 (vaginal). 42, 43 and 43 years. For the GOG cases, the age range was 25-81 y (mean 48 y, median 47 y), and for the subset with no stated exposure to DES the age range was 25-81 y (mean and median 50 y). Overall 6 of the 7 DES women had carcinomas associated with stromal mucin versus 29/98 GOG cases overall ($p = 0.002$), and 16/43 of patients W 45 y ($p = 0.016$). In the 1 DES case in which stromal mucin was absent in a small biopsy specimen, abundant stromal mucin and viable tumor was present in an irradiated hysterectomy specimen. The mean age of the GOG cases with stromal mucin was 44.3 y versus 49.9 y for those in which it was absent ($p = 0.049$). For the GOG cases with a reported negative history of DES exposure, stromal mucin was present in only 6/20 cases ($p = 0.01$), and in 5/8 patients W 45 y ($p = NS$).

Conclusions: Based on a small number of cases, cervical and vaginal non-CCCa in DES exposed women appears to be characterized by a mucinous epithelial element with stromal mucin. All of the patients were < 45 y, and 5/7 were 40-43 y. Among non-selected cases of cervical non-CCCa from GOG protocols, the presence of stromal mucin was borderline significantly associated with a younger age. One possible explanation for the presence of stromal mucin in DES exposed patients is their young age, but overall, DES was significantly associated with stromal mucin when compared with the GOG patients W 45 y. In most of the GOG cases with stromal mucin in women W 45 y, no mention of DES exposure is reported, and in the remaining cases, it is probable that no detailed investigation was undertaken, since many gynecologic oncologists pursue a history of DES exposure only in cases of clear cell carcinoma. Consequently, some of the GOG cases of stromal mucin in younger women may have also been associated with DES. While the number of cases is small, the results suggest that stromal mucin in cervical and vaginal non-CCCa in women W 45 y may be a potential marker for DES exposure. However, a larger study must be undertaken to determine whether mucinous carcinoma with stromal mucin is significantly associated with DES. If a significant link is established between DES and cervical and vaginal non-CCCa with stromal mucin, patients with DES exposure will require continued close monitoring after the risk of cervical clear cell carcinoma has diminished.

DES-exposed People Need Research

Pat Cody, Program Director, DES Action USA

DES Action's 4-panel poster illustrates the importance of research to the DES-exposed community, the relevance of animal models to human outcomes, and the pressing concerns of consumers.

The first panel is entitled *DES-exposed People Need Research.* The headlines Immune System? Menopause? Third Generation? DES Sons? and Questions from our Members, which includes excerpts of letters from DES-exposed individuals, highlight the most urgent concerns of the DES-exposed population.

Sentinels & Sensors: The DES-exposed Human Offspring uses an Escher-like illustration to show the connections between animal and human research. The headings reflect major areas for research, and are as follows: Breast Cancer, Immune System, Clear-Cell Cancer, Reproduction, Male Infertility, and Bones. Each heading lists two studies, one involving laboratory animals, and one involving humans. The poster is designed to show the importance of DES as a model of an environmental agent with estrogenic potential, as well as the relevance of basic laboratory research to the human population.

Opinion Leadership and Health Behavior: A Randomized Community Trial from the National DES Education Program

Deborah L. Wingard[1], Barbara A. Cohn[2], June Flora[3], Rhiannon Patterson[2], Caroline Shooler[3], Nora Cody[4], Pat Cody[4] and Susan P. Helmrich[5]. [1]University of California San Diego, [2]Public Health Institute, [3]Stanford University, [4]DES Action, [5]DES Cancer Network.

The goal of this trial was to increase awareness and confirmation of diethylstilbestrol (DES) exposure among at-risk women, using an innovative intervention which educates community leaders to diffuse information through naturally occurring social networks. Random cross-sectional population surveys were conducted in randomly selected intervention and control communities of 50,000, before and two years after the 1995 intervention (N≈450 women in each sample). Media and physician awareness campaigns were conducted in both communities. In the intervention community, ≈100 women identified as “opinion leaders” were trained to spread DES information via their social networks. Multivariate logistic regression analysis revealed an intervention effect (p=0.055) on the confirmation behavior of the highest-risk women and on the frequency of DES discussion in the community (p=0.007). The odds of a woman having confirmed her DES exposure increased by 60% in the intervention community (p=0.018). DES discussions increased by 70% A concomitant physician intervention was insufficient to promote diffusion of positive health behavior in the community at-large. The success observed with this intervention for an issue of low salience suggests that it has great potential to address numerous other health issues. Cost-effectiveness of this social network intervention makes it promising for community-wide behavior change.

Results of a Community Intervention Trial to Educate Health Providers on Care of DES-exposed Patients: Report from the National DES Education Program

Barbara A. Cohn[1], Piera M. Cirillo[1], Deborah L. Wingard[2], Steve J. McPhee[3], B. Gerbert[3], Pat Cody[4] and Nora Cody[4]. [1]Public Health Institute, [2]University of California San Diego, [3]University of California San Francisco, [4] DES Action.

This is a preliminary report of the effectiveness of a randomized, controlled, community-based intervention to educate health providers about the care of patients exposed to diethylstilbestrol (DES) based on the California site of the National DES Education Program. DES is a synthetic estrogen given to women during pregnancy between 1938 and 1971 which was later shown to be ineffective. DES exposure is associated with cancer in mothers and daughters and with reproductive anomalies in daughters and sons. This study tested the hypothesis that academic detailing would increase provider knowledge of DES and encourage providers to take a DES history. Providers were surveyed before and after receiving the intervention. A DES knowledge score was constructed by giving equal weight to 20 items. Pretest and post-test scores were compared for 55 providers in three geographically distinct communities matched on demographics: Community I, where both providers and consumers received an intervention; Community II, where only providers received an intervention, and Community III (control). Analyses examined the association of post-test scores with intervention condition adjusted for baseline scores using multivariate linear regression (DES knowledge) or logistic regression (DES-history taking). Preliminary results showed a significant increase in DES knowledge in Community I vs. the control ($p=0.01$). There was also a trend towards increased DES knowledge in Community II ($p=0.13$). The proportion of providers who take a DES history routinely increased in Community II ($p=0.03$) and Community I ($p=0.06$) relative to the control community. Findings suggest that academic detailing can increase knowledge of DES and that provider knowledge in linked to provider behavior.